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Biosynthesis of sialosyllactotetraosylceramide in human colorectal carcinoma cells

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A monosialoganglioside, IV^3-NeuNAcLcOse$_4$Cer, has recently been detected in colorectal carcinoma cells, small cell lung carcinoma cells, embryonal carcinoma cells and in human brain extracts. We report here the presence of a CMP-sialic: LcOse$_4$Cer sialyl transferase activity in subcellular membrane fractions of the human colorectal carcinoma, SW1116, which recognizes the non-reducing terminal galactosyl moiety of lactotetraosylceramide. A convenient method for structural analysis of picomolar quantities of the radioactive enzymatic product(s) using bacterial endoglycoceramidase, sialidase and a viral sialidase is presented.

Glycolipid; Ganglioside; Transferase; Sialidase; (Carcinoma cell)

1. INTRODUCTION

Following the report of a monoclonal antibody (C-50) which reacts with monosialogangliosides of the lacto-series, a glycolipid antigen was discovered in lung carcinoma cells defined by the structure IV^3-NeuNAcLcOse$_4$Cer [1]. Subsequently it has also been reported to be present in embryonal carcinoma cells [2] and in minor quantities in the human brain [3]. We have previously reported the biosynthesis of Lewis^a, Lewis^b and H-1 fucolipids, via fucosylation of lactotetraosylceramide, by enzymatic activities in colorectal carcinoma Golgi and post-Golgi subcellular fractions [4]. The purpose of the present study was to demonstrate sialylation of lactotetraosylceramide via transferase activity in human colorectal carcinoma cell (SW1116) membranes which directs *N*-acetylneuraminic acid to the non-reducing terminal galactosyl moiety.

Our results are the first to describe an $\alpha 2 \longrightarrow 3$ sialyl transferase for lactotetraosylceramide of documented purity and structure, which is responsible for the biosynthesis of IV^3-NeuNAcLcOse$_4$Cer, the putative precursor of the 19-9 glycolipid antigen [5]. We also demonstrate that under controlled incubation conditions bacterial and viral sialidases are useful for structure analyses of gangliosides in picomolar quantities, where chemical modification is impractical.

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Abbreviations: HPTLC, high performance thin-layer chromatography; Mab, monoclonal antibody; IV^3-NeuNAcLcOse$_4$Cer, NeuNAc($\alpha 2 \longrightarrow 3$)Gal($\beta 1 \longrightarrow 3$)GlcNAc-($\beta 1 \longrightarrow 3$)Gal($\beta 1 \longrightarrow 4$)GlcCer (Nomenclature of glycolipids according to the IUPAC-IUB commission on biochemical nomenclature [(1977) Eur. J. Biochem. 79, 11–21])

2. MATERIALS AND METHODS

2.1. *Materials*

SW1116 cells were cultured in serum-free media, harvested and washed as described [6]. Lactotetraosylceramide was kindly provided by Dr Göran Larson (Dept of Clinical Chemistry, Sahlgren's Hospital, University of Göteborg, Sweden). Authentic IV^3-NeuNAcLcOse$_4$Cer and C-50 MAb were kindly provided by Drs Lars Svennerholm and Pam Fredman (Dept of Neurochemistry, Sahlgren's Hospital, University of Göteborg, Sweden). Authentic IV^6-NeuNAcnLcOse$_4$Cer was kindly provided by Dr Olle Nilsson (University of Göteborg). Endoglycoceramidase from *Rhodococcus* was a generous gift of Dr T. Yamagata (Mitsubishi Company, Glycoconjugate Research Division, Japan). Influenza sialidase was obtained through the good offices of Dr J.J. Skehel (National Research

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Institute, Mill Hill, London, England). Bacterial sialidases were obtained from Boehringer-Mannheim (FRG). CMP-[^{14}C]sialic acid (154–351 μCi/μM) was purchased from Amersham (England) and CMP-sialic acid was purchased from New England Nuclear (Boston, MA, USA).

2.2. *Methods*

A Golgi and post-Golgi membrane fraction (ϱ 1.16–1.18) from SW1116 has been characterized by techniques reported [4].

2.2.1. Standard assay for radioactive IV^3-NeuNAcLcOse$_4$Cer

70 μg of Na^+ taurocholate was dissolved with 5 μg LcOse$_4$Cer in $CHCl_3/CH_3OH$, 2:1, in a conical assay tube. Other components of the incubation were (final concentrations): cacodylate (0.15 M), sucrose (0.3 M), $MgCl_2$ (1 mM), CDP-choline (0.5 mM), $MnCl_2$ (1 mM), CMP-[^{14}C]sialic (100 μM) and 6–10 μg membrane protein, all in a total volume of 50 μl, pH 6.3. Incorporation of radioactivity into monosialoganglioside was linear up to 4 h.

2.2.2. Analysis of the radioactive enzymatic products

Incubations were terminated by the addition of 0.5 ml of $CHCl_3/CH_3OH$ (3% H_2O) and allowed to sit overnight at 4°C to precipitate protein and sucrose. After the addition of 1 μg of GM1 ganglioside (Supelco, PA, USA) the extracts were dried down to 25 μl and spotted on 3 MM Whatman paper for ascending chromatography in 1% Na^+ borate, pH 9.1, for 90 min.

After the chromatogram was completely dried, the origins were excised and extracted again with $CHCl_3/CH_3OH/H_2O$, 60:35:8, v/v, 3 times each, 2 ml. These extracts were applied to DEAE-Sepharose (acetate) columns (1 × 0.5 cm) which were eluted subsequently with 3 ml, $CHCl_3/CH_3OH/H_2O$, 60:35:8 and 7 ml of 0.03 M $NH_4^+OAc^-$ in methanol. The methanol fraction was dried down and counted in 0.5 ml methanol and 10 ml Insta-gel (Packard) against minus glycolipid controls in a LKB Beta-Rack programmed counter.

2.2.3. Neuraminidase specificity assays

Each incubation contained about 500 dpm of ^{14}C-labelled enzymatic product, 7.5 μg taurodeoxycholate, 250 μU endoglycoceramidase in a total volume of 20 μl buffered with 0.05 M Na^+ acetate (pH 5.5), incubated at 37°C for 90 min. 20 μg of IV^3-NeuNAcLcOse$_4$ (Biocarb, Lund, Sweden) were then added and 50 μg of viral sialidase or 10 μU *V. cholerae* in 5 μl of 0.05 M acetate, and the incubating continued for up to

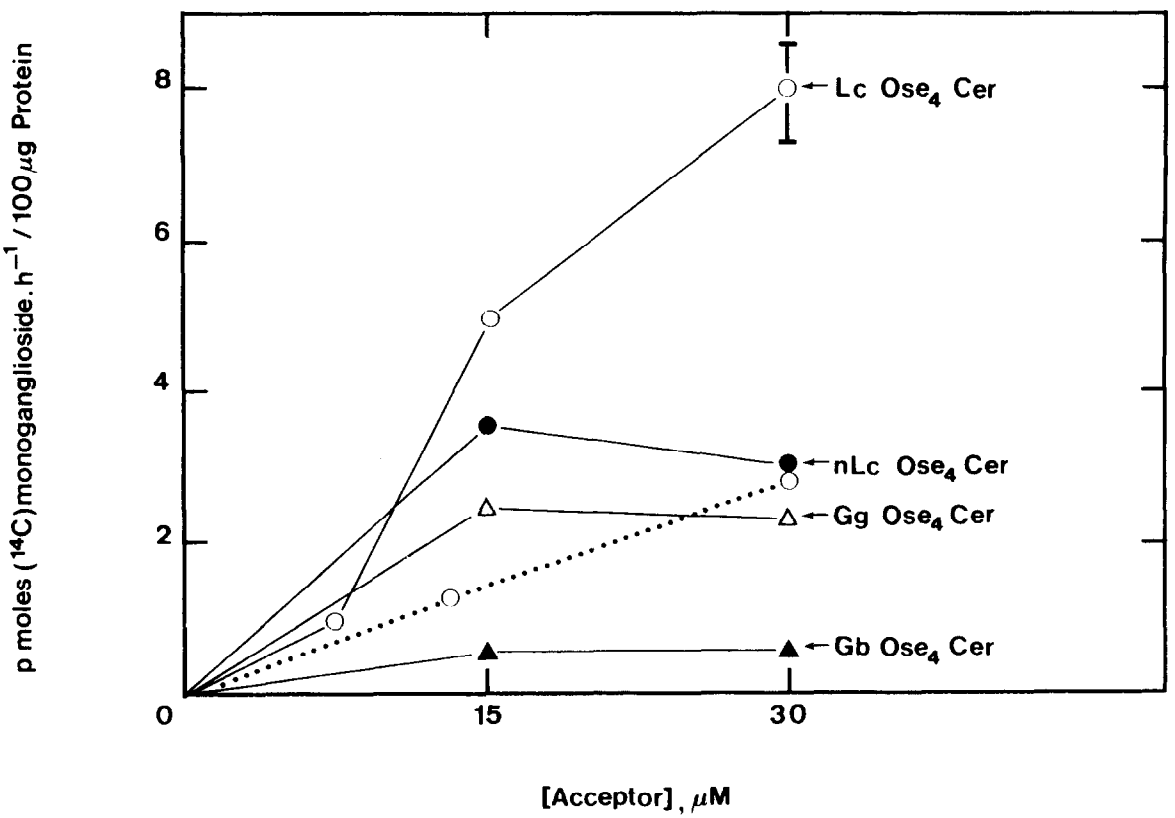

Fig.1. Incorporation of ^{14}C from CMP-[^{14}C]sialic into monosialoganglioside fractions as a function of glycolipid acceptor concentration. (o---o) Galactose oxidase treated LcOse$_4$Cer [4].

1 h. Control incubations contained III^6-NeuNAcLcOse$_4$ or IV^6-NeuNAcnLcOse$_4$ (Biocarb). The products were analysed by HPTLC on Merck nanoplaque Si 60 plates in the system butanol/AcOH/H_2O, 2:1:1, v/v and by resorcinol spray for oligosaccharide standards and fluorography for the radioactive products [6].

3. RESULTS AND DISCUSSION

Of the 4 'core' structures tested, LcOse$_4$Cer, nLcOse$_4$Cer, GgOse$_4$Cer and GbOse$_4$Cer, intact LcOse$_4$Cer was the best acceptor of sialic acid from CMP-[^{14}C]sialic in the presence of SW1116 post-Golgi membrane fractions (fig.1). Galactose oxidase pretreatment of the LcOse$_4$Cer [4] destroyed most of the acceptor capacity of the glycolipid (fig.1).

The enzymatic activities responsible for the sialylation of other acceptors may be due to the presence of other transferases in these membrane fractions, or to the presence of an $\alpha 2 \longrightarrow 3$ transferase which does not have an absolute specificity for the sugar sequence between the terminal non-reducing galactosyl moieties and the ceramide. Sialylation of GgOse$_4$Cer to form IV^3-NeuNAcGgOse$_4$Cer has been recently reported [7].

Previous work with a sialyl transferase which synthesizes IV^3-NeuNAcnLcOse$_4$ in submaxillary gland and Pc9 lung carcinoma cells appears to indicate specificity for the nLcOse$_4$ (type 2) sequence [8,9] and does not sialylate LcOse$_4$.

Further purification and characterization of sialyl transferases from colorectal carcinoma cells are in progress to resolve this issue.

The migration of the monosialoganglioside radioactive product on HPTLC plates in the system $CHCl_3/CH_3OH$/2.5 M NH_3 is shown in fig.2. The major product comigrates with authentic IV^3-NeuNAcLcOse$_4$Cer just ahead of II^3-NeuNAcGgOse$_4$Cer and not with IV^6-NeuNAcnLcOse$_4$Cer (fig.2).

Further evidence for the structure of the major radioactive enzymatic product was obtained using an assay coupling endoglycoceramidase (EGCE) [10] and sialidases from Influenza and *V. cholerae* [11]. EGCE will not release sugars from glycoproteins. We observed about a 60% release of the oligosaccharide. Subsequent viral and bacterial sialidase activity released up to 90% of the sialosyl moiety under conditions which left over 81% of the IV^6-NeuNAcnLcOse$_4$ and III^6-NeuNAcLcOse$_4$ pentaoses intact.

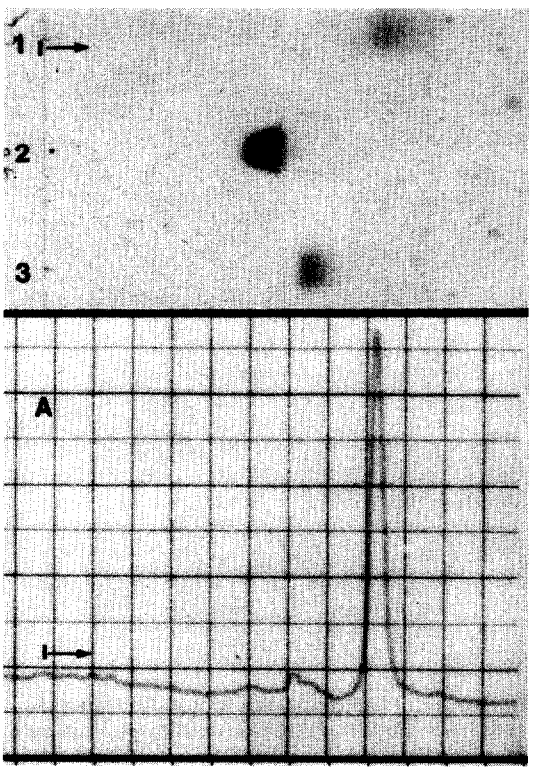

Fig.2. Migration of authentic monosialoganglioside on HPTLC in relation to the ^{14}C-labelled enzymatic product of LcOse$_4$Cer sialylation, in the solvent system $CHCl_3/CH_3OH$/2.5 M NH_3, 60:40:9. Lanes: 1, IV^3-NeuNAcLcOse$_4$Cer; 2, IV^6-NeuNAcnLcOse$_4$Cer; 3, II^3-NeuNAcGgOse$_4$Cer; A, densitometer tracing of a fluorogram of the ^{14}C-labelled enzymatic product after chromatography.

To our knowledge this is the first demonstration of the $\alpha 2 \longrightarrow 3$ sialylation of the terminal galactosyl moiety of lactotetraosylceramide of documented purity and structure [12]. Our results should be useful for determination of this enzymatic activity in tissues where monosialogangliosides of the lacto-series are either exiguous or cryptic. One reason why there is some inconsistency in the evidence for the notion of 'tumor associated' glycolipid antigens is that some of them are the limiting substrates for further glycosylation and hence do not accumulate sufficiently for detection by Mab. The appropriate transferases may, nonetheless, be detectable in vitro.

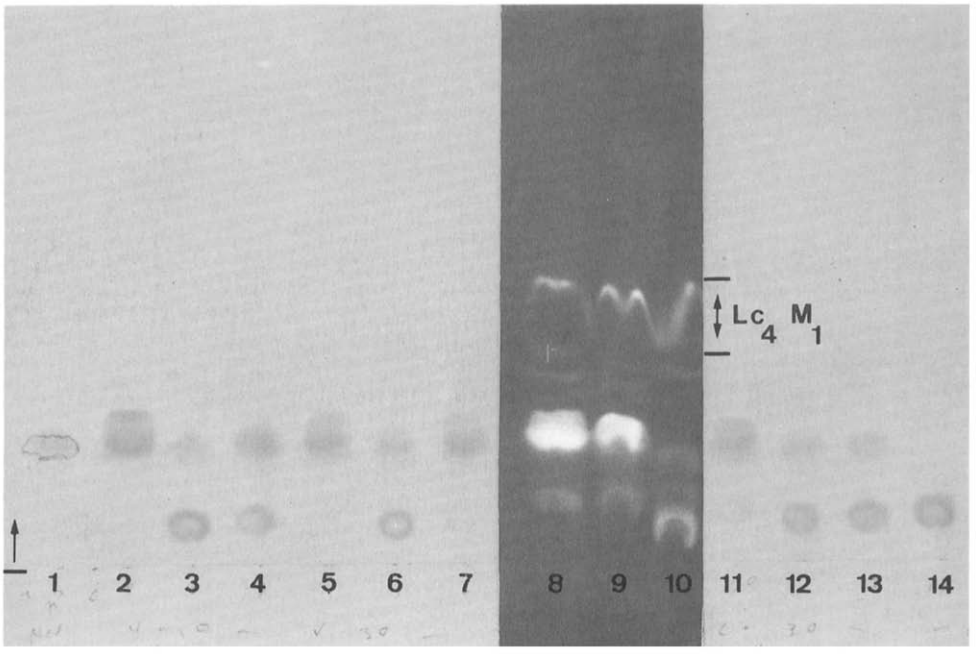

Fig.3. Migration of authentic pentaoses and *N*-acetylneuraminic acid (NANA) in relation to the ^{14}C-labelled enzymatic product treated with endoglycoceramidase (EGCE) and sialidases. Lanes: 1, authentic NANA; 2–7, influenza viral sialidase (10 and 30 min) incubation with authentic IV^3-NeuNAcLcOse$_4$ (2 and 5), III^6-NeuNAcLcOse$_4$ (3 and 6), IV^6-NeuNAcnLcOse$_4$ (4 and 7); 11–13 show results with *V. cholerae* incubation for 30 min with IV^3-NeuNAcLcOse$_4$ (11), III^6-NeuNAcLcOse$_4$ (12), IV^6-NeuNAcnLcOse$_4$ (13); 8, ^{14}C-labelled enzymatic product treated with EGCE and viral sialidase, 10 min; 9, ^{14}C-labelled product treated with EGCE and *V. cholerae* sialidase, 30 min; 10, ^{14}C-labelled product treated with EGCE only; 14, authentic pentaose treated with buffer only.

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